

# Antimicrobial properties of a new polymeric material based on poly(2-hydroxyethyl methacrylate)

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**Abstract.** *Background and aim.* One of the promising areas is the development of synthetic wound dressings with programmed release of active substances that can affect various elements in the pathogenesis of the wound process. The aim was to study the antimicrobial properties of a new polymeric material based on poly(2-hydroxyethyl methacrylate). *Methods:* 2-hydroxyethyl methacrylate, dimethacrylate triethylene glycol as crosslinking agent, polymerization initiator of azobisisobutyronitrile along with a porogen and one of the antimicrobial agents, including decamethoxin, chlorhexidine bigluconate, silver nitrate, octenidine, furacilin, metronidazole, dioxidine, and gentamicin were used to synthesize a new material with antimicrobial activity. For comparison, polymer samples synthesized without adding antimicrobials were used, as well as known dressing materials: activated carbon material, porcine skin, which were immersed into 0.02% decamethoxin solution before use, as well as silver-containing dressing, hydrogel dressings, including those filled with silver. Determination of antibacterial properties was performed by diffusion method. *Results.* Low antimicrobial activity of the studied existing wound dressings, which are widely used in medical practice, even under conditions of their saturation with antiseptic substances, has been established. Samples of the suggested polymeric material with the addition of antimicrobial substances showed the ability to inhibit the growth of the test strains of microorganisms at a sufficient level, especially with such fillers as decamethoxin, gentamicin, dioxidine. When metronidazole was added to the polymeric material, a reliable antimicrobial effect on the anaerobic microorganisms was established. *Conclusions.* Modification of the polymeric material of poly(2-hydroxyethyl methacrylate) by adding antimicrobial substances allows to ensure its high antimicrobial properties against different microorganisms. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** Wound Infection, Local Antimicrobial Agents, Wound Dressings, Poly(2-hydroxyethylmethacrylate), Controlled Release.

## Introduction

The problem of wounds remains relevant in surgery until today and is far from being solved (1). Over

the years, treatment theories have changed many times. This was mainly against the background of advances in the study and understanding of the etiopathogenetic features of the wound process (2, 3). As a result, new

methods and means have emerged to correct it (4). One such achievement was the development of wound dressings, which actually initiated a new phase in the local treatment of wounds (5). With further growth of this area, wound dressings began to appear, which differed in the chemical composition of the base and the drugs that were part of them (6). In the modern pharmaceutical market there are hundreds of different wound coatings that can affect various elements of the wound process pathogenesis (7). However, their main purpose is effective antimicrobial action, as well as protection of the wound from drying and mechanical trauma, stimulation of reparative processes, and usability to prevent possible adhesion of the coating material to the wound surface (8). Bandages with interactive properties have become a promising vector for the development of this direction (9). This became possible due to the emergence of a new generation of polymeric materials with appropriate structural, physical and mechanical properties, as well as the widespread introduction of nanotechnology. Such materials include poly(2-hydroxyethyl methacrylate) (PHEMA), which is widely used in various fields of medicine: surgery, neurosurgery, ophthalmology, and combustiology (10, 11). Recently developed ways to modify the structure of this material, combination with other polymeric bases, and the possibility of filling with biologically active substances have significantly expanded its useful properties (12, 13). In particular, we have suggested a new polymeric material based on the hydrogel PHEMA, synthesized with the addition of a porogen and antimicrobial substances.

Studies of physicochemical properties of the suggested material confirmed its mechanical strength and stability (14). Pharmacokinetic analysis established its ability to prolonged controlled release of antimicrobial substances into the aqueous medium. The introduction of different amounts of a porogen can change the pore size, which, in turn, allows to adjust the pharmacokinetic parameters of the material (15). Given the success of the results, it is advisable to conduct a microbiological study of the material, which includes studying the effectiveness of its action on reference and clinical strains of opportunistic pathogens. Purpose of the study was to study the antimicrobial properties of a new polymeric material based on poly(2-hydroxyethyl methacrylate).

## Materials and methods

Liquid monomer 2-hydroxyethyl methacrylate (Sigma-Aldrich, Germany), triethylene glycol dimethacrylate (Sigma-Aldrich, Germany) as crosslinking agent and azobisisobutyronitrile (Sigma-Aldrich, Germany) as polymerization initiator were the initial compounds for synthesis of new material. Prior thermopolymerization, a porogen (distilled water) and one of the antiseptic or antibacterial drugs were added to this mixture: decamethoxine (Pharmchim, Ukraine), chlorhexidine bigluconate (Viola, Ukraine), silver nitrate (Macrochem, Ukraine), octenidine (Schülke & Mayr GmbH, Germany), nitrofurazone (Menadiona S.L., Spain), metronidazole (Hubei Hongyuan Pharm. Co., China), dioxidine (Farmak, Ukraine) or gentamicin (Galychpharm, Ukraine). The resulting material had the form of a mechanically strong translucent gel. For comparison, polymer samples synthesized without adding antimicrobials were used, as well as known dressing materials: activated carbon material (Dnipro, Ukraine), lyophilized porcine skin xenograft (Institute of Biomedical Technologies, Ukraine), which were immersed into 0.02% decamethoxine solution for 30 min before use, as well as silver-containing ointment dressing (Hartmann, Germany), hydrogel dressings, including those filled with silver (ConvaTec, UK).

Determination of antibacterial properties of the studied materials in the form of disks with a diameter of 6 mm and a thickness of 2 mm was performed by agar diffusion method [16]. To this end, a layer consisting of thoroughly mixed nutrient agar medium was poured into a Petri dish with daily testing of the purity of the culture of studied microorganism (n=12). Reference strains (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606) and clinical (*Staphylococcus aureus* 8, *Pseudomonas aeruginosa* 71, *Acinetobacter baumannii* 56) of opportunistic microorganisms were used in the experiments. The microbial load amounted to  $10^8$  colony-forming unit (CFU) per 1 ml, which was established according to the McFarland standard. Mueller-Hinton agar (Sanimed-m, Ukraine) was used for the study.

Additionally, the ability of the samples to affect anaerobic strains of microorganisms with a microbial load of  $10^8$  CFU per 1 ml of medium was studied (17).

The culture of *Clostridium perfringens* 28 was grown according to the Zeissler's method: Kitt-Tarozzi medium with inoculated culture was heated at 80 °C for 20 min to destroy vegetative and preserve spore-forming microorganisms. Culture tubes were filled with sterile vaseline oil and placed in a thermostat for 24 hours at 37 °C. After 24 hours of cultivation the microorganisms were inoculated on sugar nutrient agar with the addition of D-cyclodextrin and methylene blue at a concentration of 1:20000 to prevent diffusion of oxygen into the medium and cultured in a thermostat for 24 hours. Thioglycolate medium with the addition of kanamycin 100 mg/l or vancomycin 7.5 mg/l, phytomenadione 1.5 mg/ml, hemin 10 mg/l and bile salts 100 mg/l was used to grow asporogenic anaerobic microorganisms. The study involved the use of modified thioglycolate medium (Farmaktiv, Ukraine) under conditions of limited access to oxygen. Cultivation of asporogenic anaerobic microorganisms was performed on thioglycolate medium with the addition of nalidixic acid 1.5 µg/ml, kanamycin 100 mg/l or vancomycin 7.5 mg/l for *Peptostreptococcus anaerobius* 13; kanamycin 100 mg/l or vancomycin 7.5 mg/l, phytomenadione 1.5 mg/ml, hemin 10 mg/l, bile salts 100 mg/l for *Bacteroides fragilis* ATCC 13/83; kanamycin 100 mg/l or vancomycin 7.5 mg/l, phytomenadione 1.5 mg/ml, hemin 10 mg/l, diamond green solution 0.0002% for *Fusobacterium necrophorum* 22.

Evaluation of antimicrobial activity was performed a day after according to the degree of growth retardation in the area of application of the test composition.

The following criteria were used for this purpose: the absence of growth retardation zones of microorganisms around the well with test sample, as well as a delay of up to 10 mm which indicated lack of sensitivity of the microorganism to the tested drug; growth retardation zone with a diameter of 10–15 mm indicated moderate resistance of the culture to the studied concentration of antibacterial substance; growth retardation zone with a diameter of 15–25 mm was regarded as an indicator of the sensitivity of the microorganism to the studied drug substance; growth retardation zone of more than 25 mm indicated a high sensitivity of the microorganism to the studied drugs (18).

The obtained results were processed statistically using data processing software Microsoft Excel 2016 and "Statistica 5.5" (licence № AXXR910A374605FA).

## Results

Analysis of the results showed that the samples of polymers filled with decamethoxine, gentamicin, dioxidine have a sufficiently high antimicrobial activity against all studied opportunistic pathogens with a growth retardation diameter for *S. aureus* ATCC 25923 in the range of 20.77±0.21 mm, 27.95±0.31 mm, 18.33±0.22 mm, respectively, *P. aeruginosa* ATCC 27853 – 18.57±0.27 mm, 26.89±0.29 mm, 21.38±0.34 mm, respectively, *A. baumannii* ATCC 19606 – 22.27±0.29 mm, 30.69±0.36 mm and 28.76±0.33 mm, respectively.

**Table 1.** Antibacterial activity of the studied samples against reference strains of *S. aureus*, *P. aeruginosa*, *A. baumannii*

Studied materials		Diameters of growth retardation zone, mm (n=12)		
		<i>S. aureus</i> ATCC 25923	<i>P. aeruginosa</i> ATCC 27853	<i>A. baumannii</i> ATCC 19606
PHEMA +	decamethoxine	20.77±0.21	18.57±0.27	22.27±0.29
	chlorhexidine bigluconate	12.11±0.24	–	11.23±0.27
	octenidine	11.37±0.43	–	–
	gentamicin	27.95±0.31	26.89±0.29	30.69±0.36
	dioxidine	18.33±0.22	21.38±0.34	28.76±0.33
Xenograft + decamethoxine		10.83±0.45	–	–

Notes: PHEMA - poly(2-hydroxyethyl methacrylate); "–" – no growth retardation zone.

In the study of other materials, growth retardation zones could not be established or they did not exceed 10 mm in diameter.

Samples impregnated with a solution of chlorhexidine bigluconate showed weak activity against *S. aureus* ATCC 25923, forming a growth retardation zone within  $12.11 \pm 0.24$  mm and *A. baumannii* ATCC 19606 –  $11.23 \pm 0.27$  mm.

The low antimicrobial effectiveness was found in polymeric material containing octenidine and a lyophilized xenograft saturated with decamethoxine: among studied microorganisms only *S. aureus* ATCC 25923 demonstrated low sensitivity to the mentioned antiseptics (diameters of growth retardation zone were  $11.37 \pm 0.43$  mm,  $10.83 \pm 45$  mm, respectively) (Table 1).

Additionally, the sensitivity of clinical strains of the same microorganisms to the samples, that showed the highest antimicrobial activity in the previous study – the suggested polymeric materials containing decamethoxine, gentamicin and dioxidine. The obtained results confirmed high antimicrobial properties of materials with decamethoxine and gentamicin (Table 2). Samples containing dioxidine were not effective enough and were accompanied by signs of secondary

growth in growth retardation zone around the material (Fig. 1).

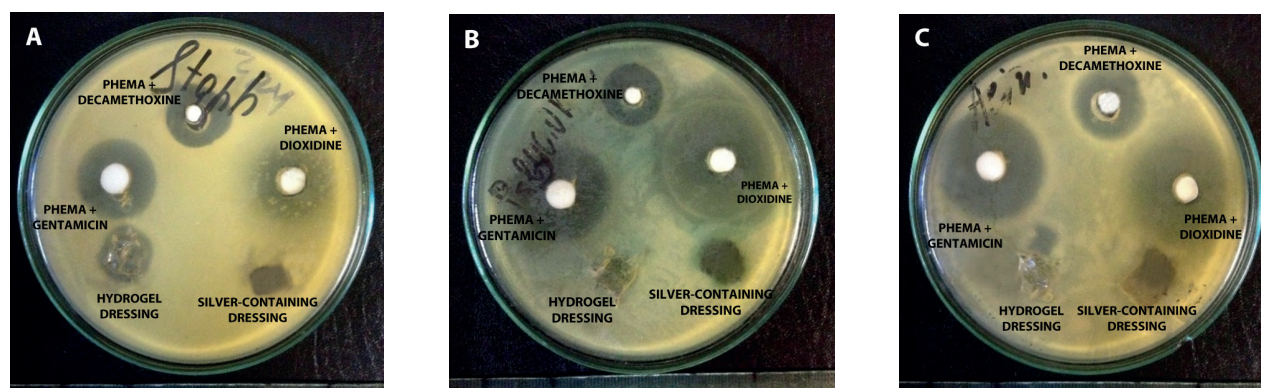
Results of the study of the sensitivity of anaerobic microflora to antimicrobials allowed to establish the effectiveness of metronidazole in the polymeric material with growth retardation of microorganisms *C. perfringens* 28 at  $17.73 \pm 0.21$  mm, *P. anaerobius* 13 –  $20.11 \pm 0.27$  mm, *B. fragilis* ATCC 13/83 –  $18.94 \pm 0.35$  mm, *F. necrophorum* 22 –  $19.65 \pm 0.29$  mm in diameter.

Thus, the results obtained in this study provided the opportunity to define the feasibility of adding an antiseptic solution of decamethoxine and the antibacterial drug gentamicin to the polymeric material PHEMA in order to provide it with antimicrobial activity. There are currently discussions about the safety of topical antibiotics (19). Some cases of successful topical use of aminoglycosides in infectious processes of different locations are compromised by reports not only of their negative impact directly on tissues, but also of the ability to cause severe systemic toxic lesions and the formation of antibiotic resistance in

**Table 2.** Antibacterial activity of the studied samples against clinical strains of *S. aureus* 8, *P. aeruginosa* 71, *A. baumannii* 56

Studied materials		Diameters of growth retardation zone, mm (n=12)		
		<i>S. aureus</i> 8	<i>P. aeruginosa</i> 71	<i>A. baumannii</i> 56
PHEMA +	decamethoxine	$20.48 \pm 0.34$	$14.59 \pm 0.25$	$20.43 \pm 0.32$
	gentamicin	$27.13 \pm 0.19$	$25.75 \pm 0.31$	$28.63 \pm 0.37$
	dioxidine	Secondary growth		

Note: PHEMA - poly(2-hydroxyethyl methacrylate)



**Fig. 1.** Results of the study of antimicrobial activity of the studied samples against clinical strains of *S. aureus* 8 (a), *P. aeruginosa* 71 (b), *A. baumannii* 56 (c).

Note: PHEMA - poly(2-hydroxyethyl methacrylate)



microorganisms (20-22). Therefore, any form of antibiotics for topical application – irrigation, applications, impregnation of suture or dressing materials, etc. – are not widely recommended by researchers. Limited effect of materials with metronidazole only on the anaerobic microflora also significantly narrows the scope of their practical use. In general, the introduction of antimicrobial agents into the polymer matrix, from which they are gradually released, can be considered as a promising approach to creating safe and effective means for local wound treatment (23).

## Conclusions

Modification of the polymeric material poly(2-hydroxyethyl methacrylate) by adding antimicrobial substances allows to ensure its high antimicrobial properties against Gram-positive, Gram-negative aerobic and anaerobic microorganisms. Polymers containing decamethoxine possess optimal properties, which makes them a promising material for the development of effective wound dressings.

**Conflict of Interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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